Development of Value-Added Products from Sago Frond

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ABSTRACT

Sago frond is the compound leaf of the palm *Metroxylon sagu*. Our earlier studies revealed that this material can be harnessed to produce Sago Frond Sugar (SFS), which contains cellobiose and glucose as the main sugars at about 10g/L and 5g/L, respectively. SFS has been proven as the comprehensive fermentation medium in production of L-lactic acid using *Lactococcus lactis* IO-1. The yield and productivity of L-lactic acid from SFS is comparable to the Standard Medium (even when amended with yeast extract) at 0.85g/g and 85%, respectively. SFS can be purified using Powdered Activated Charcoal (PAC) to produce Purified Sago Frond Sugar (PSFS) and has been shown to have antibacterial properties against several foods related bacteria. Cellobiose from sago frond was perceived to be beneficial as an antifungal agent when tested against *Candida* which is a common cause of skin infection, where the growth of *C. tropicalis* is highly affected by SFS. However, this purification process distinctly reduces its antifungal properties. In our attempt to increase the sugar concentration (hence the enzymatic activities), SFS was heated to yield 50% and 100% sugar concentrations. However, these too reduce its antifungal effect drastically by 20% and 0%, respectively. Sago frond sap, the liquid obtained by pressing freshly de-skinned sago fronds on a roller press machine, has been fermented to produce ethanol using the yeast *Saccharomyces cerevisiae*. The sap contains a higher amount of sugar (25 g/L) compared to SFS (15 g/L). It was observed that sterilised SFS exhibited cell growth of up to 90% of the yeast biomass. Astonishingly, similar growth was obtained when raw (unsterilized) sap was used as the fermentation medium. This confirmed our conclusion that sago frond sap can be used directly as the medium in large-scale ethanol fermentation without the need for sterilisation. Recently, sago frond has also been utilised for the production of silage as animal fodder. A combination of leaves and frond fibres can generate 42 tons of silage from the harvesting of approximately 500 sago logs, every day. In our preliminary trials, the best practice is to mix shredded sago leaves with sago frond sap which enhances the lactate fermentation using *Lactococcus lactis* IO-1 to produce nutritious silage. *L. lactis* IO-1 was recycled as the inoculant to increase the fermentation efficiency and quality of sago frond silage.

Keywords: sago fronds, sago leaves, cellobiose, lactic acid, ethanol, silage, animal feed.

INTRODUCTION

This is a review paper, covering previous works on production of lactic acid from prebiotic sugars obtained by enzymatic hydrolysis of pulverised sago frond fibres. The sugar, termed Sago Frond Sugar (SFS) contains mostly cellobiose together with some glucose. Direct fermentation of SFS without further amendment has been achieved with considerable success on the yield of L-lactic acid, an important commodity with various uses. From here, our on-going research continues on the use of cellobiose due to its antibacterial and antifungal properties. The latest development is the use of raw sago frond sap as the medium for ethanol fermentation, generating a high amount of biomass when it is sterilised and amended with yeast extract.

It has been postulated that by the year 2050 food production needs to be amplified by as much as 60% to meet global demand due to speedy expansion of the human population (FAO, 2012). Unfortunately for some countries, fertile land is getting scarce and clean water sources are diminishing. Ignoring this target will certainly affect our world in terms of food security, stability and general peace (Konuma, 2018). Alternative source of food from the hardy, underutilised and unpretentious sago palm that generates starch at 20-25 ton/ha (Ishizaki, 1997), suddenly becomes very interesting. Since the average annual intake of starch per person is approximately 250 kg, it has been postulated that a 1,000ha sago farm can support and save 100,000 people from starvation (Ishizaki, 1997).

The largest sago palm growing areas (wild and semi-cultivated) is Indonesia, followed by Papua New Guinea. But the largest systematically cultivated sago estate (approx. 50,000 ha) supplying logs to modern factories that extracts, dries and packs totally white food-grade sago starch in just over 30 minutes upon rasping is in Sarawak, Malaysia. Since the year 2000, Sarawak has established new sago plantations mainly in the districts of Mukah, Dalat, Igan, and Pusa. However, the long delay (10-12 years) prior to harvesting together with a minimum of other saleable products from sago farms are two of the biggest challenges to expand the sago industry.

This paper focuses on multiple uses of the sago fronds to augment the income of farmers between and after harvesting of the sago palms. In the district of Mukah in Sarawak, approximately 500 sago palms are harvested daily to feed the eight large factories, each one generating between 20 to 30 tons of starch/day. At this rate, and at about 10-15 fronds/palm, a minimum of 5,000 fronds with a total weight of 30 tons is discarded into the environment each day, unutilised (Ahmad, 2015). We have revealed that enzymatic hydrolysis of sago frond pith can produce cellobiose, a potential substrate in production of lactic acid (Ahmad, *et al*., 2016). Our current work focuses on sago frond sap which has been shown to be highly fermentable by yeast to produce ethanol.

1. PREBIOTIC SUGAR

Prebiotics are known as beneficial food components which are indigestible by the host and enhance the growth and activities of specific bacteria populations in the colon. Thus, the bacterial growth enhanced by the presence of prebiotics will improve human health. All kinds of prebiotic are made up of fibre but not all fibres are prebiotic. The properties of the prebiotic

substance are unable to be hydrolysed by human enzymes, resist gastric acid, and not be absorbed by the upper gastrointestinal tract. The prebiotic component must be able to be fermented by colonic microflora and able to stimulate the activity and growth of colonic bacteria associated with positive effect on human health and well-being (Slavin, 2013).

Due to lack of the enzyme cellobiase (β-glucosidase) in the human body, cellobiose cannot be hydrolysed into glucose for the absorption of human cell because of the glucose-glucose β (1-4) linkage (Stephen, 2014). Thus, cellobiose is categorised as a non-edible sugar. However, some of the probiotic bacteria that live in the human colon do have the ability to absorb and consume cellobiose as a carbon source for the growth and production of beneficial by-products such as lactic acid and butyric acid (Nippon Paper Industries, 2016).

According to Basholli-Salihu et al (2013) supplementation of cellobiose in the fermentation of milk by using *Bifidobacterium infantis* suggests that cellobiose is efficient as a prebiotic ingredient in fermented products involving bifidobacteria. According to Adeni et al (2018), the production of cellobiose was carried out by utilising the cellulose component of sago frond to produce a significant amount of combination between cellobiose and glucose by using cellulase enzyme originating from *Trichoderma reesei*. The specific enzyme was selected due to the low composition of the β-glucosidase enzyme in the mix that is responsible to break down cellobiose into glucose; hence the concentration of cellobiose can be maintained at the maximum level. At optimum enzymatic hydrolysis conditions, cellobiose can be produced at 16% (g/g) recovery rate with 60% conversion efficiency.

The product of enzymatic hydrolysis of sago frond is justified as Sago Frond Sugar (SFS). In addition to cellobiose, SFS also possessed flavonoid and phenolic compounds at 0.423 g/L and 1.86 g/L respectively. To study the prebiotic effect of cellobiose, purification of SFS need to be conducted using powdered activated charcoal can increase the purity of cellobiose but totally wipe out the valuable flavonoid and phenolic as antioxidant compounds to prevent the compound from compromising the growth performance of the selected probiotic strain (Ahmad, 2017).

The *Lactococcus lactis* sp. was used as an indicator strain to study the prebiotic effect of cellobiose from purified SFS. The result indicates that *L. lactis* cultured in SFS produces significantly higher total biomass compared to standard medium (SM). The yield of *L. lactis* growth in SFS is higher than SM exhibiting the better efficiency of cellobiose as the carbon source than glucose for the cultivation probiotic. Analysis on the growth profile of *L. lactis* in SFS shows enhanced viability where the strain continuously grows further than the normal incubation period. Both of the conditions suggest that cellobiose act as slow-release carbon source; as a matter of fact, cellobiose is protected by a β-glycosidic bond making it more complex to be metabolised by specific bacteria, especially by the probiotic in the human digestive system implying the potential of cellobiose as a prebiotic supplement for human health (Ahmad et al, 2020).

2. NATURAL ANTIBIOTIC AGAINST FOOD-BORNE ASSOCIATED DISEASE BACTERIA

Purified Sago Frond Sugar (PSFS) produced by filtration on Powdered Activated Charcoal (PAC) has been shown to have antibacterial properties against several foods related bacteria such as *Staphylococcus aureus*, *Escherichia coli* and *Salmonella typhi* (Ahmad, 2014). Antimicrobial agents developed from organic or natural material are useful in order to meet the demand of newer drugs that are safe to use. It was reported that the cellobiose derivative has the highest antimicrobial activity against the bacterium *E. coli* (Antonio *et al.,* 2010).

Concentrated sugars are commonly acknowledged as food preservatives used to prolong the shelf life of food by creating osmotic pressure to prevent the manifestation of undesired microorganisms. However, excessive sugar intake can compromise the nutritional benefit of the food and is commonly related to obesity, diabetes and heart diseases. Hence, non-fermentable sugar such as cellobiose was proposed as an alternative to preserving food considering the similar capability to manipulate osmotic pressure as fermentable sugar (Kunz et al. 2012). Despite being used as a preservative, non-fermentable sugar can potentially be developed as a natural antibiotic to fight against food-borne associated disease bacteria.

Purified sago frond sugar possessed a high concentration of cellobiose used as the stock solution to study the antibiotic properties of the SFS. The result shows that SFSis highly effective in inhibiting the growth of gram-negative indicator strains represented by *Staphylococcus aureus* and *Escherichia coli* and followed by *Salmonella typhi* as a gram-positive indicator strain. However, *Listeria monocytogens* was completely unaffected by the SFS (Ahmad et al, 2023).

Complete genome analysis of *S. aureus* and *S. typhi* reveals the absence of a gene encoding a Phosphotransferase System (PTS) cellobiose protein acceptor. Both strains are incapable of transporting cellobiose into the cell via the correct channel in the absence of PTS, while *E. coli* single gene expression site is insufficient for efficient cellobiose translocation. Both situations are plausible catalysts for glucose repression, which prevents the cell from consuming glucose and ultimately results in carbon starvation. Similarly, a high concentration of cellobiose during the initial growth stage inhibits sporulation and, consequently, the viability of *C. cellulilyticum*, while a low concentration of cellobiose prevents the induction of proteolytic activities essential for sporulation (Gehin et al., 1995). Bacteriocin inhibits the nutrient assimilation of bacteriocin-intolerant strains via a similar inhibition mechanism (Waite and Hutkins, 1998) that is also exhibited by bacteriocin produced by lactic acid bacteria. A high concentration of cellobiose may induce a competitive inhibition with glucose, thereby preventing glucose uptake and causing cell mortality by starvation. *L. monocytogenes*, on the other hand, possessed four PTS gene expression sites across its entire genome, indicating the strain's superior ability to transport cellobiose into the cell system and metabolise it as an internal carbon source. This condition, coupled with the discovery of the *bglA* gene in *L. monocytogenes*, indicates that the strain's ability to produce β-glucosidase completely nullified the inhibitory effect of cellobiose in the PSFS.

All three indicator strains inhibited by PSFS are classified as bacteria associated with foodborne illness. These genotypes are all present in the human digestive tract. Consequently, there is a strong likelihood that PSFS can be used as antibacterial agents to treat bacterial infections that can cause food poisoning (Ahmad et al, 2023).

3. ANTIFUNGAL CLEANSER

In this study, cellobiose was tested on *Candida* sp to observe its potential against fungal infection. Preliminary analyses showed that both SFS and purified sago frond sugar (PSFS) affects only *C. tropicalis* and not *C. albicans*, exhibited as the absence of inhibition zone on agar plates. Meanwhile, SFS was highly effective against *C. tropicalis* with an average clearing zone of 9mm. This was somewhat reduced to 7mm when it was heated and concentrated to 50% in our attempt to increase its antifungal properties. PSFS have lesser activity against *C. tropicalis* at a clearing zone of lower than 6mm which disappears altogether when concentrated to 50%. High concentration increases the viscosity of the sago frond sugars and reduces its penetration into the agar, hence decreasing antifungal activities.

4. LACTIC ACID

Lactic acid is widely used in a host of industries such as food and beverages, cosmetics (skin care, toiletries and hair care products), pharmaceutical and various industrial applications including textile, chemical, metal and cleaning agents. However, only L-lactic acid can be used in beauty products due to its complete absorption and compatibility with the human skin. Meanwhile, D-lactic acid is an endogenous compound under certain circumstances that can be harmful if exposed through spoilt food and beverages or pathogenic microbiota infestation (Pohanka, 2020). Pure L-lactic acid can be produced by microbial fermentation using the lactic acid bacteria *Lactococcus lactis* IO-1 due to its ability to produce only L-lactic acid (Ishizaki and Okta, 1989; Jolhiri and Bujang, 1998; Bujang *et al*, 2000).

Versatile properties of lactic acid ideal for application in various industries such as food, pharmaceutical, cosmetic, bioplastic, and medical established it as one of the most important and valuable organic acids in the global market. The global lactic acid market size forecast is worth USD 3.7 billion in 2020 and expected to increase up to USD 8.7 billion in revenue by 2025. The compound annual growth rate (CAGR) of lactic acid was projected at 18.7% from 2019 to 2025 (Global View Research, 2019). The outbreak of the Covid-19 pandemic creates a huge positive impact on the global demand of lactic acid and polylactic acid for the manufacturing of food packaging and personal protection equipment (PPE) due to the protective effect of lactic acid against pathogenic microorganisms escalated the global demand of lactic acid (Markets and Markets, 2020). Lactic acid is manufactured through chemical synthesis and biotechnological fermentation of materials obtained from the food-based crop. An increase in the usage of food-based crops raises an ethical concern about the usage of food resources to produce chemicals and biofuel that may compromise global food security and safety, especially for poor and developing countries. Hence, alternative raw materials such as agricultural by-products to produce lactic acid must be prioritised to ensure a sustainable global food supply.

According to Ahmad et al. (2020), sago fronds exhibit optimistic properties to be exploited as feedstock for lactic acid production. Due to the increasing demand for sago starch, a large number of sago palms are harvested, resulting in the accumulation of sago fronds abandoned

in the sago farm. Complex lignocellulosic structure slows down the degradation process of the sago frond and can cause an environmental problem if improperly managed. However, enzymatic hydrolysis of the lignocellulosic compound using cellulase enzymes produced a substantial concentration of sugar that can be converted into lactic acid. The low composition of β-glucosidase in the cellulase enzyme produced by *Trichoderma reesei* caused cellobiose as the major sugar followed by glucose. Due to the complex structure of cellobiose, only selected bacterial strains were able to metabolise cellobiose and glucose to lactic acid simultaneously. *Lactobacillus delbrueckii* mutant Uc-3 have the ability to utilise both cellobiose and cellotriose efficiently, for the production of lactic acid. The mutant converts 90 g/L of lactic acid from 100 g/litre of pure cellobiose with 2.25 g/L/hour productivity (Adsul et al, 2007). Application of *L. lactis* IO-1 as inoculum managed to break the β-1,4 glycosidic bond of the cellobiose which releases two molecules of glucose that can be directly converted into lactic acid. In addition, the complex structure of cellobiose acts as a slow-release carbon source allowing proper carbon utilisation management by the *L. lactis* IO-1 give opportunity for the strain to be more viable and increase maximum growth of the bacteria; thereby, suggesting cellobiose from sago frond express prebiotic effect on the growth of *L. lactis* IO-1.

Sago frond contains large quantities of lignocellulose components (15.8% hemicellulose, 41.4% cellulose and 6% lignin). Enzymatic hydrolysis of dried sago frond powder using cellulase enzymes produces Sago Frond Sugar (SFS), made of cellobiose (16-18%) and glucose (9-11%). SFS also contains Mg (72.66 mg/L), Mn (18.37 mg/L) and Cu at 0.04 mg/L which are needed by Lactic Acid Bacteria (Ahmad*,* 2017).

The potential of SFS for the production of lactic acid was analysed on three types of fermentation media; Standard Medium (SM, made of commercial glucose and yeast extract at 4:1 ratio), Sago Frond Sugar amended with yeast extract (SFS+YE) and only Sago Frond Sugar (SFS). As expected, the production of lactic acid was highest from the SM medium (22.81g/L) with 94% sugar consumption and 94% lactic acid recovery, obtained after 42 hours (**Table 1**). The comparable yield was observed in SFS+YE medium at 21.40g/L and 96.2% for the production of lactic acid and sugar consumption, respectively. This was achieved 6 hours later, at 48 hours. However, it was observed that the SFS medium produced only slightly lower yield of lactic acid at 19g/L within the same duration (48 hours), reflecting the advantage and economics of using SFS as it is for future large-scale processes in reducing fermentation costs.

Table 1: Production of Lactic Acid and sugar consumption from Standard Medium (SM), Sago Frond

	Fermentation Media		
Parameters	SM	SFS+YE	SFS
Lactic acid (g/L)	22.81g/L	21.40g/L	18.64g/L
Residual sugars (g/L)	0.85	0.91	4.23
Sugar consumption (%)	93.8	96.2	84.8
LA recovery (%)	94.2	89.2	80.8

Sugar amended with Yeast Extract (SM+YE) and only Sago Frond Sugar (SFS).

As expected, slightly higher (5%) yield of lactic acid (0.90g/g) and other kinetic parameters (productivity 0.54g/L/hr, efficiency 90.8%) was evident when using Standard Medium (SM) compared to the other two media, being the complete medium with glucose as the main carbon source (**Table 2**).

Table 2: Kinetic parameters in production of L-lactic acid from Standard Medium (SM), Sago Frond Sugar amended with Yeast Extract (SFS+YE) and Sago Frond Sugar only (SFS).

	Fermentation Media		
Parameters	**SM**	**SFS+YE**	**SFS**
Lactic Acid Yield (g/g)	0.90±0.005	0.85±0.006	0.85±0.001
Productivity (g/L/hour)	0.54±0.003	0.45±0.009	0.39±0.001
Efficiency (%)	90.81±0.53	85.03±0.63	84.76±0.85

Only slightly lower yield at 0.85g/g, productivity at 0.45g/L/hr at 85% efficiency was observed in the medium SFS+YE. The obvious advantage is that the yield and efficiency of the process in only SFS medium is still the same at 0.85g/g and 85%, respectively. This confirmed that SFS can be fermented into lactic acid as it is with comparable outcomes to the Standard Medium (SM).

Meanwhile, sago fronds can produce sago frond sap. Sago frond sap contains glucose as the primary sugar, followed by xylose. As stated by Kato et al. (2012), *L. lactis* IO-1 is a versatile lactic acid bacterium that can convert glucose and xylose into lactic acid by using different pathways. The competency of *L. lactis* IO-1 to conduct direct fermentation on free sugar from the sago frond juice can reduce the production cost by the elimination of enzymatic hydrolysis from the process flow of the lactic acid production. Therefore, sago frond was an ideal alternative raw material to produce L-lactic acid on account of sustainably cheap raw material, and the simple processing method also denies the accusation of ethical issues regarding the application of food-based feedstock to produce lactic acid.

The sap can be extracted easily from sago frond using a roller-press machine. It contains free sugars, primarily glucose (43.8 g/L) and xylose (17.3 g/L), together with residual starch (5.55 g/L). Enzymatic hydrolysis followed by sterilisation can maximise sugar yield rendering it suitable as the substrate for L-lactic acid fermentation. The ability of *L. lactis* IO-1 to metabolise both glucose and xylose promoted the sugar consumption up to 89.93% while amendment with salt and neutralizing agent allowed *L. lactis* IO-1 to effectively consume sugar higher than normal threshold of 30 g/L. This encourages growth of biomass to the maximum which later can be used as the inoculant in silage manufacture (Ahmad *et al*, 2022). The application of fermentation produces not only high quality and pure lactic acid but also cultivates superior LAB that can be developed as a probiotic strain or effective microorganism (EM) that can be used in various applications such as food supplementation to prevent gastrointestinal disease in human or animal. EM is commonly used in the agricultural industry to improve the production of compost fertiliser, stimulate the growth performance of plants by reconditioning the soil and use it as an inoculant to improve the quality and enhance the stability of silage for animal feed to support the growth of livestock industry.

5. PROBIOTIC CULTIVATION MEDIA

Probiotics are defined as biological agents that offer a positive health effect to the host by bringing balance to the microflora population and activity. Lactic acid bacteria (LAB) are generally associated with probiotic bacteria due to its capability to produce substantial lactic acid that can be used against pathogenic bacteria that are commonly acid intolerant. Lactic acid bacteria are conventionally found in fermented food to enhance the flavour and prolong the storage time of the product. Due to its versatile capability, the cultivation of lactic acid bacteria is expanded to other applications such as cosmetic, medical, and agriculture.

As claimed by Park (2018), ProBionic Corp. successfully formulated and developed a skincare product containing living probiotic bacteria known as *Lactobacillus sakei* proBio65 strain exhibit the inhibitory effect against atopic dermatitis and psoriasis-like skin lesions and showed promising efficiency as the anti-inflammatory agent through oral and skin topical administration.

Lactic acid bacteria demonstrate several health and nutritional benefits from selected species. The LAB enhances food nutritional value, prevents intestinal infection, promotes digestion of lactose, manages some types of cancer, and acts as a serum to control cholesterol levels (Gilliland, 1990). Fermentation technology was established as an essential process in food manufacturing and has been practised for centuries to produce culture-influenced food such as ‘Tempe’ by the Javanese, ‘Kimchi’ by the Korean and ‘Miso’ by the Japanese. The active compound produced through the fermentation process of the food possessed a positive effect on health by reducing the risk of heart disease, indigestion, boosting immunity and weight loss (Coyle, 2020). According to Othman et al. (2019), a preliminary study shows that *L. lactis* M4 exhibits the ability to penetrate cancer cells, specifically to colorectal cancer. The *L. lactis* M4 is an ideal vector to transmit plasmid DNA into cancer cells to trigger bactofection so the cell can express the gene encoding for heterologous protein (antigens, toxin or enzyme) to invade the cancer cell from inside.

Even though the viability of LAB in the gastrointestinal and skin of living organisms provides a positive effect on the host, LAB can also be found in soil and on the leaf's surface. Domination of LAB is important to improve soils, prevent disease and enhance plant performance commonly associated with bio-fertiliser. The LAB act as plant growth promoter bacteria (PGPB) to enhance the solubility of the fertiliser to improve the efficiency of the nutrient absorption by the plant, and LAB act as a biocontrol agent that offers effective protection against a wide spectrum of phytopathogenic fungal and bacterial infection. The application of immature compost can lead to massive production of ammonia gas but can be neutralised by LAB cultivation (Lamont et al., 2017).

On the other hand, fermentation of the ingredient to produce animal feed with the reinforcement of LAB improves the nutritional value and extends the life span of the animal feed. According to Sugiharto and Ranjitkar (2019), fermented feed demonstrates a positive effect on the gastrointestinal ecosystem and morphology, immune function, and act as a growth promoter to the poultry. Introducing LAB to the poultry system through a feed delivery mechanism allows the LAB to colonise the digestive system to lower the pH by producing substantial lactic acid to reduce pathogenic microorganism infestation. In addition, LAB is found naturally in silage

and inoculation of selected LAB strain in the production of silage was proven able to improve the quality of the animal feed for the ruminant.
In order to cultivate the probiotic bacteria as such lactic acid bacteria require specifically formulated media that possess optimum carbon source associated with ideal nutrients and supplements required by the selected strain to grow effectively. Hence, sago frond sap has been designed as fermentation media to fulfil the explicit requirement of *Lactococcus lactis* sp.

6. HAND SANITISER

This hand sanitizer (registered as SaFrond) was developed from L-lactic acid produced from fermentation of sago frond sugar. Selective antibacterial properties of SaFrond protect the skin from pathogenic bacteria while other beneficial bacteria indigenous to the skin is not affected, hence creating a balance of microflora on our skin. SaFrond hand sanitizer is non-toxic due to the organic ingredient, hence completely harmless should it be accidentally consumed. It uses organic fragrance (phthalates free) from pandan extract (*Pandanus amaryllifolius*) and free from any preservatives. SaFrond hand sanitizer is completely Halal due to the absence of alcoholic contents and thus attracts a larger portion of consumers (Ahmad *et al.*, 2017b). The main advantage of this project is that it minimises disposal of sago fronds, both from pruning and harvesting. Once commercialised, this product can generate extra income to the sago farmers.

7. ETHANOL

Ethanol has been studied before this using sugar from pure sago starch (Adeni and Bujang, 1998; Bujang *et al*, 2000) but starch being a source food, it is not ethical as a substrate for large-scale production of energy.

A young sago frond (skinned) in the middle circle of the crown weighs about 5-6 kg, slightly heavier than old ones. Around 1200ml of sap can be extracted from the whole frond, hence a calculated ratio of about 1kg frond to 200ml sap. Since each sago palm harbour between 10-15 fronds, a minimum of 12L sap/palm can be procured, or a total of 6,000L from an estimated harvest of 500 palms/day in the Mukah District in Sarawak.

Due to our hot climate, the amount of sap is highly affected by the duration after harvesting and a loss of about 25% is typical after 24 hours. The sap is greenish yellow in colour (not dissimilar to sugar cane juice) and has an acidic pH between 5.0 to 5.1. It contains a higher amount of glucose (25.18 g/L) compared to sago frond fibre (15 g/L), an important criterion in large-scale ethanol or lactate fermentation.

Preliminary trials on growth of yeast (*Saccharomyces cerevisiae*) on neat sago frond sap is shown in **Figure 1**. On its own, only some increase (25%) in biomass was observed when SFS was autoclaved, which does not justify the need for it to be sterilised.

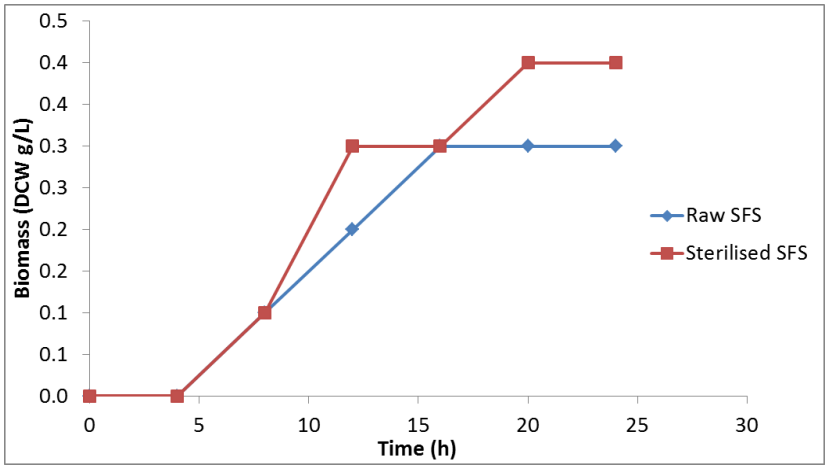

Figure 1: Effects of sterilisation on biomass of yeast in raw sago frond sap.

However, addition of 5% w/v yeast extract (YE) generates a 90% difference in the amount of biomass (as Dried Cell Weight, DCW) on raw SFS, after 24 hrs of growth **(Figure 2).** Clearly, the sap does not have to be autoclaved in our trials, addition of YE is sufficient to increase the biomass, and the subsequent production of ethanol.

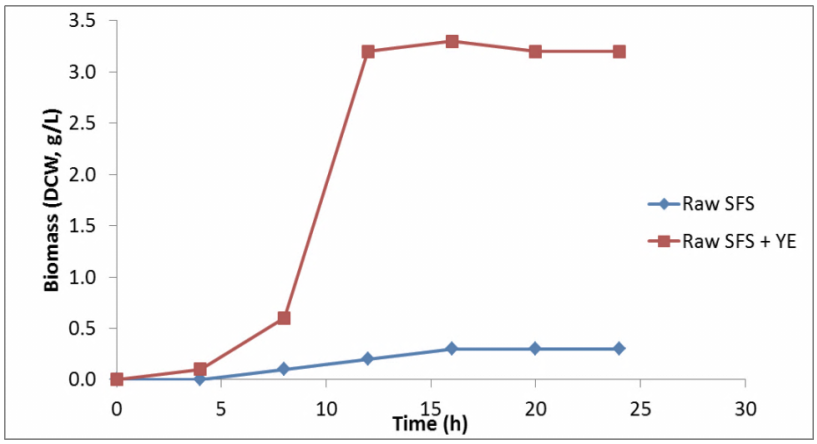

Figure 2: Effects of yeast extract on biomass of yeast in raw sago frond sap

However, when autoclaved, addition of yeast extract produced higher biomass at over 25% (4.5 g/L, **Figure 3**) compared to 3.3 g/L when using raw or unsterilized SFS, as shown previously. Due to the cost of sterilisation for large-scale processes, it is sufficient just to add yeast extract to enhance cell growth. These preliminary trials clearly support the possibility of producing ethanol directly from sago frond sap, without the need for sterilisation.

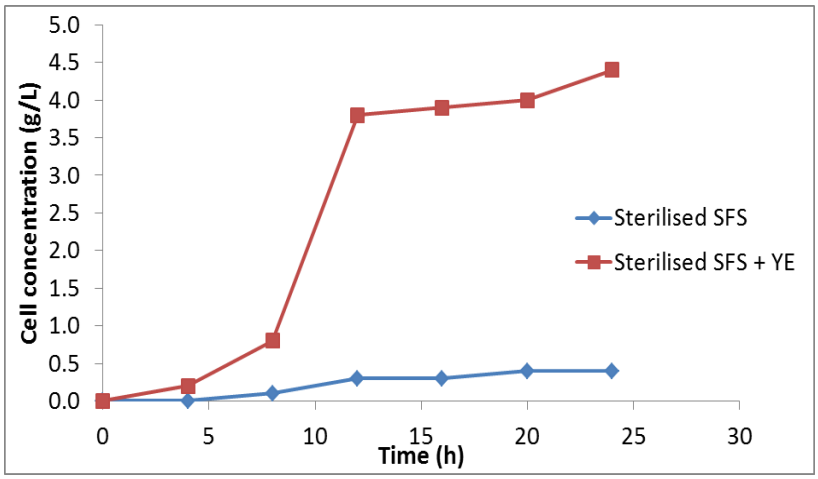

Figure 3: Effects of autoclaving and amendment with yeast extract on biomass of yeast

8. ANIMAL FEED

Silage from sago frond was prepared using leaves, preferably of the non-thorny variety. The leaves were removed from the frond, cut into small pieces (2-3 cm) and pulverised using a dry grinder. Silage from leaves is suitable for calves and young animals. In order to enhance the protein and sugar content, fibres from the frond upon extraction of the sap were added and pounded together. The mixture is promptly packed inside high-density plastic bags and vacuumed to remove air, and kept at room temperature for at least one week.

Absence of oxygen enhances the fermentation process which causes drastic degradation of protein in the silage together with excess in ammonia. This reduces the quality of the silage and severely affects the animal leading to infertility, reduces lactation, lower rumen activity and even death. Surplus production of ammonia can be avoided by enhancing the acidification process during silage fermentation (Lallemand, 2018). Using *Lactococcus lactis IO-1* (from production of lactic acid on sago frond sap) indeed enhances acidification during silage fermentation and concomitantly inhibits activity of the protease enzyme and the production of ammonia. This inadvertently will maintain quality of the silage and ensure safety of the livestock.

The 1:1 mixture of residual sago fibre (RSF) and sago leave (SL) has been determined to be the ideal formulation to produce sago frond silage which exhibit five ideal characteristics to be developed as high-quality silage from sago frond (SFSil). Inoculation of SaFLact (Effective Microorganism developed by UNIMAS Biofuel R&D Lab) improves the ensiling process by accelerating the acidification by producing a high amount of lactic acid instantly. As a result, the SFSil is better in preserving nutrient content of the silage by reducing protein degradation compared to the non-inoculated. Ironically, the extensive acidic condition of the silage due to reinforcement of SaFLact activates the lactic acid degrading bacteria. This leads to the high concentration of acetic acid at the end of the ensiling process. The production of acetic acid stabilises the pH level of the silage to maintain the viability of LAB. The substantial concentration of acetic acid improves the aerobic stability of the silage after being exposed to air, prolonging the inhibition time before yeast and mould infestation.

The feed efficiency analysis suggests that SFSil was productive for long term feed to keep up the growth performance of the growing ruminant until it reached the finisher growth stage. The autopsy on sheep fed with commercial pellet discovered excessive water accumulated in the rumen; meanwhile, the residual feed in the rumen of the sheep fed with SFSil was intact in bolus formation with the minimum amount of water influencing the moisture content and structural integrity of the faeces. Analysis of volatile fatty acid of the rumen fluid confirmed that the rapid hydrolysis of simple carbohydrates in commercial pellet leads to massive accumulation of organic acids in the ruminant responsible for the ruminal acidosis. Subsequently, ruminal acidosis reduces the efficiency of the ruminal microflora suggested that long-term feeding of a high grain diet (commercial pellet) may cause sub-acute ruminal acidosis (SARA) to the ruminant. Feeding ruminant with SFSil improves the water holding capacity of the meat exhibit the capability to maintain the structural integrity to prevent excessive water loss in the meat. The low crude fat content of meat from sheep fed with SFSil establish as low-fat meat as recognised by the Malaysian Food Act and Regulation that low-fat meat must contain less than 16% fat. High protein content and high water holding capacity do contribute to the chewiness texture of the SFSil fed meat; meanwhile, high-fat content responsible for the flavour and tenderness of the commercial fed meat influence the total preference of the panellist. Therefore, the consumer can have the option to choose between healthy or tasty meat (Ahmad, 2022).

Clearly, numerous products which have the potential to be commercialised are available to support our cash-strapped sago farmers while further developing our sago industry. The common notion of sago farming that we must wait for 10 to 12 years before we can reap what we planted is long gone. Our current studies on the use of sago leaves to generate silage and sugar from both sago starch (Bujang *et al*., 2000; Bujang, 2015) and sago fibre or *hampas* (Adeni *et al*., 2013) are achievable with lesser technologies, in tandem with minimising impact on the environment from the sago industry.

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